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Genetically-Altered Mouse Models: Short-Term Alternatives for Cancer Bioassays

- **FVB/N-TgN(v-Ha-Ras)^{Lep} (Tg.AC) Hemizygous**
- **B6.129-Trp53^{tm1Brd} (p53) Haploinsufficient**
- **B6.129-Cdkn2a^{tm1Rdp} (p16^{Ink4a}/p19^{Arf}) Haploinsufficient**





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Criteria - Short-term GMM Cancer Bioassay

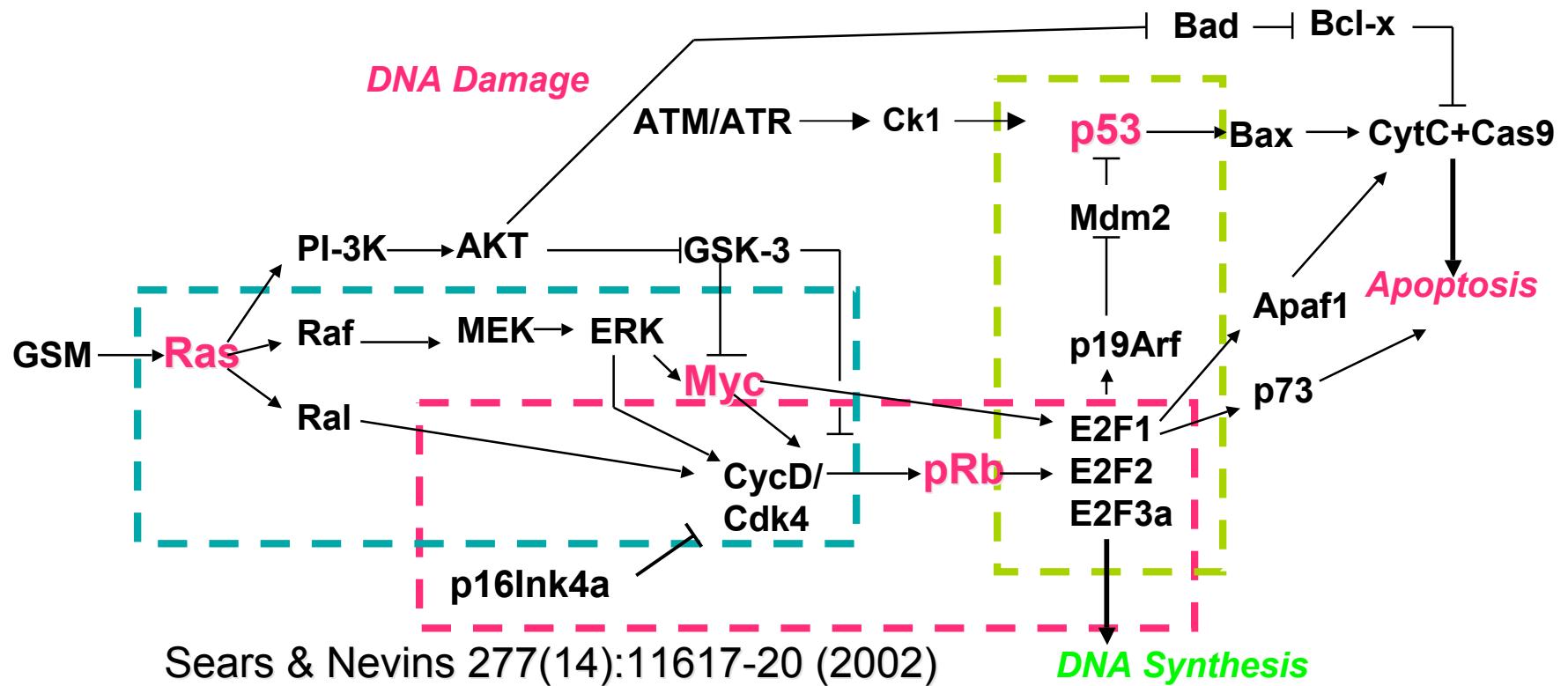
- Decreased latency (time to tumor)
- Broad range of susceptible tissues
- Zero to low incidence of sporadic tumors
- Zero to low frequency of false negatives and false positives (i.e. accurate call)
- Mode or mechanism consistent with development of human cancer



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Critical signaling interfaces





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Tg.AC Mouse

FVB/N-TgN(v-H-Ras)^{Lep}



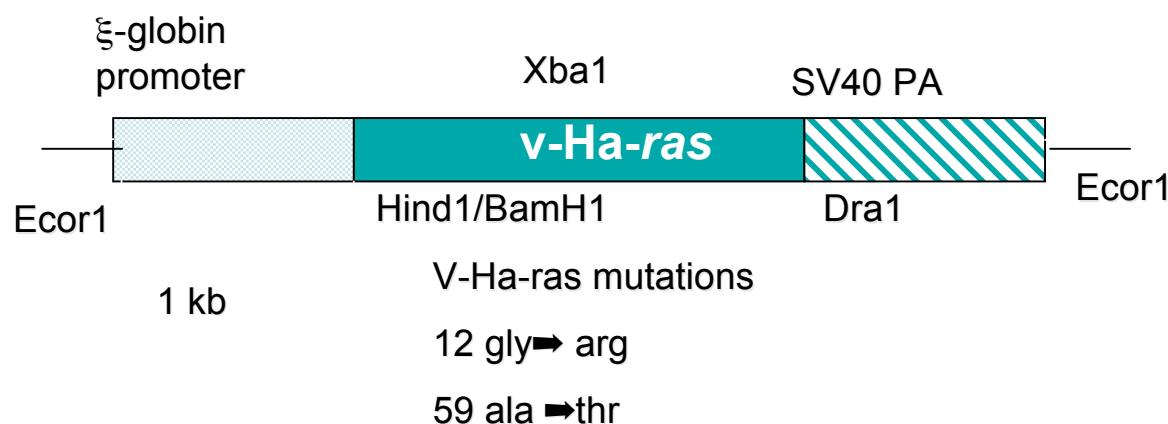


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v-Ha-ras transgene

- Tripartite construction
- Ectopic (integration site) expression
- Induced and/or clonally expanded
- Epicutaneous/Oral Exposure (Site of Contact)

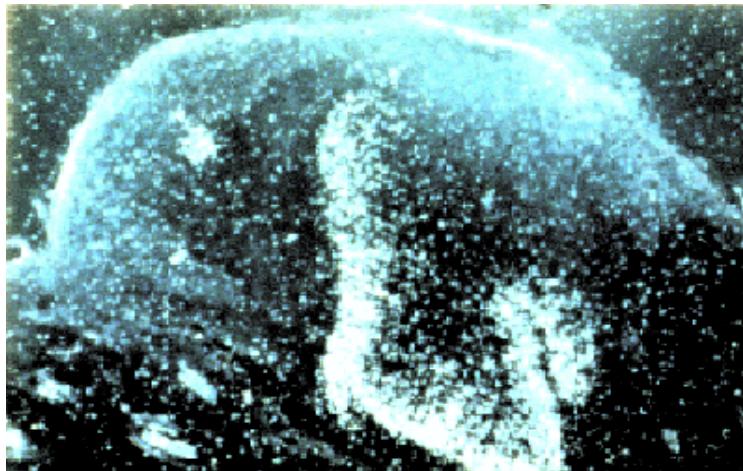




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Transgene expression (ISH)



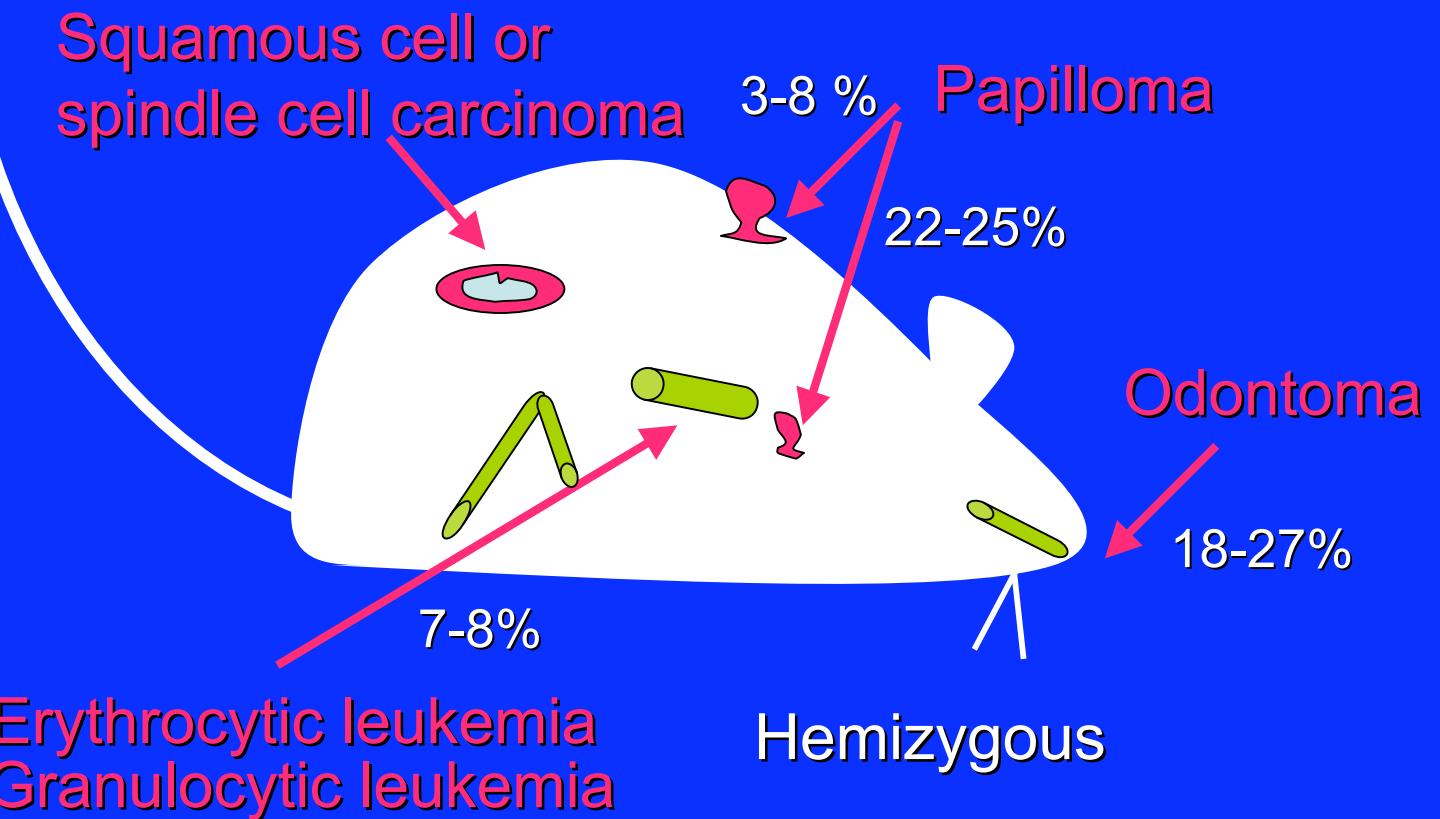
- Epidermal papilloma
- TPA induced
- Tg expression co-incident with BrdU incorporation
- Forestomach papilloma
- DMVC induced
- Tg expression co-incident with BrdU incorporation



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Spontaneous vs. Induced Tumors





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Conclusions

- Reporter phenotype (site of contact)
- Carcinogens induce and/or clonally expand cells expressing the transgene
- Carcinogens induce squamous epithelium papillomas & carcinomas



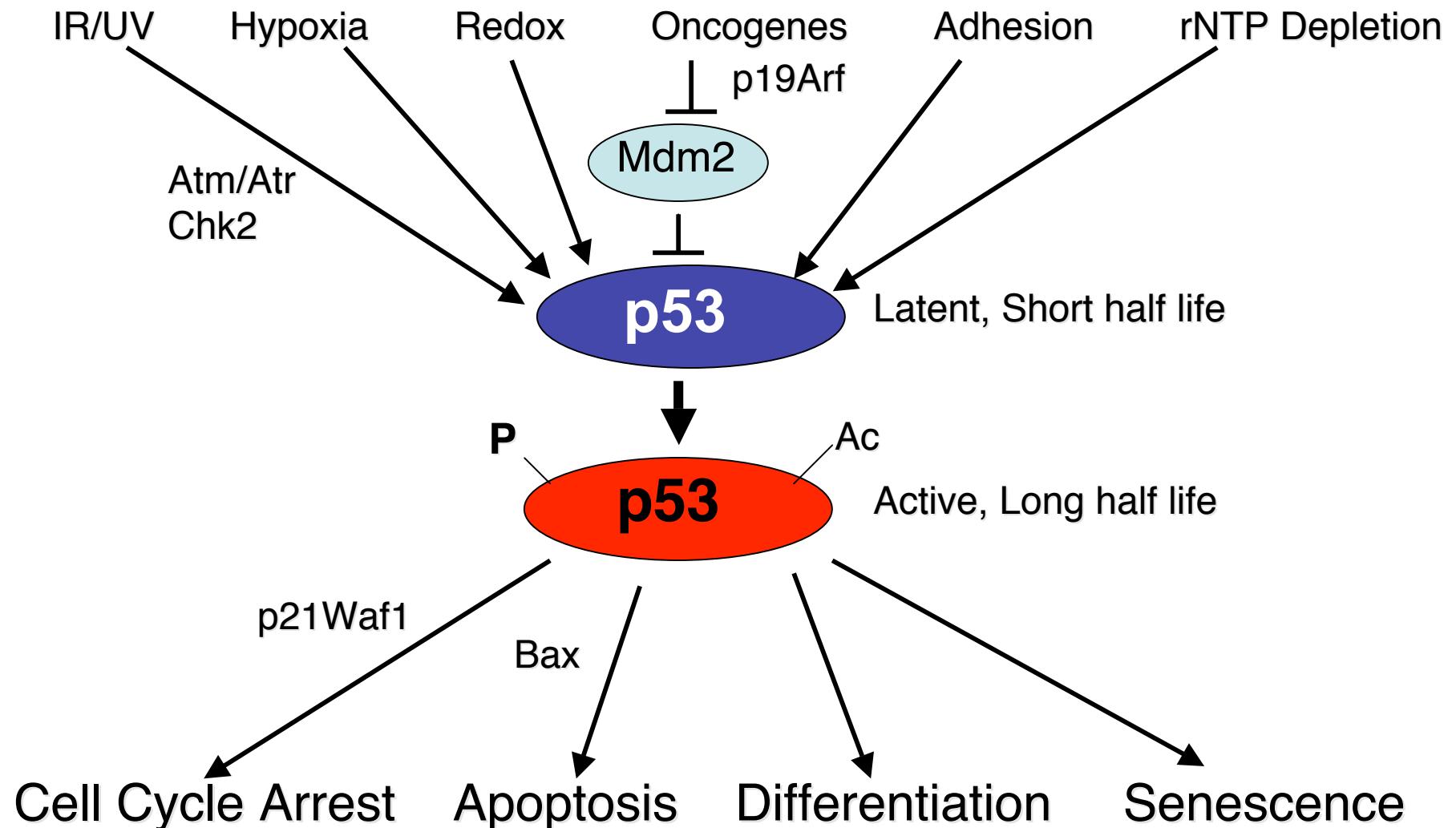
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Trp53 haploinsufficient

B6.129-Trp53^{tm1Brd} N5



Signaling through p53 gatekeeper gene



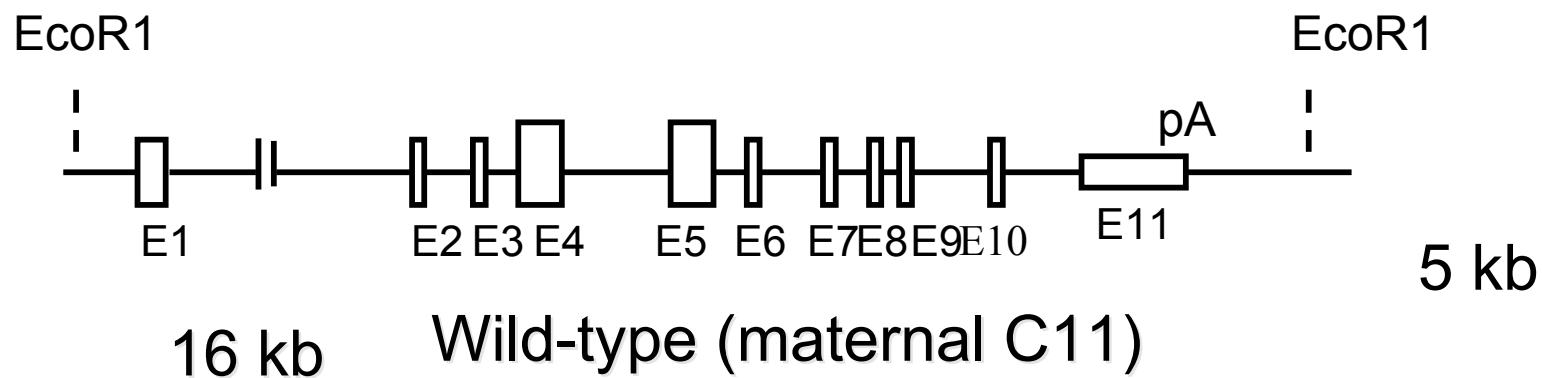
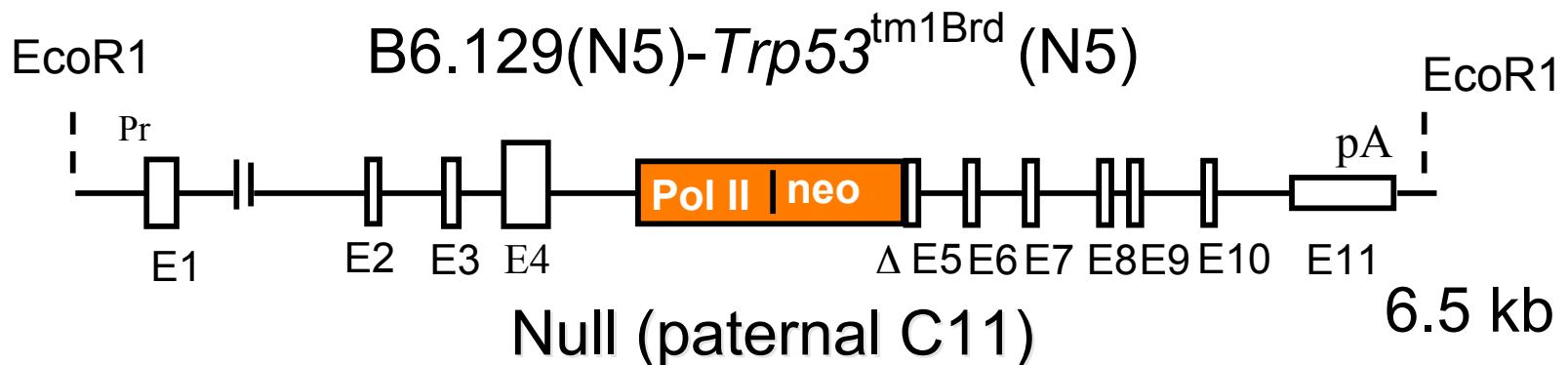


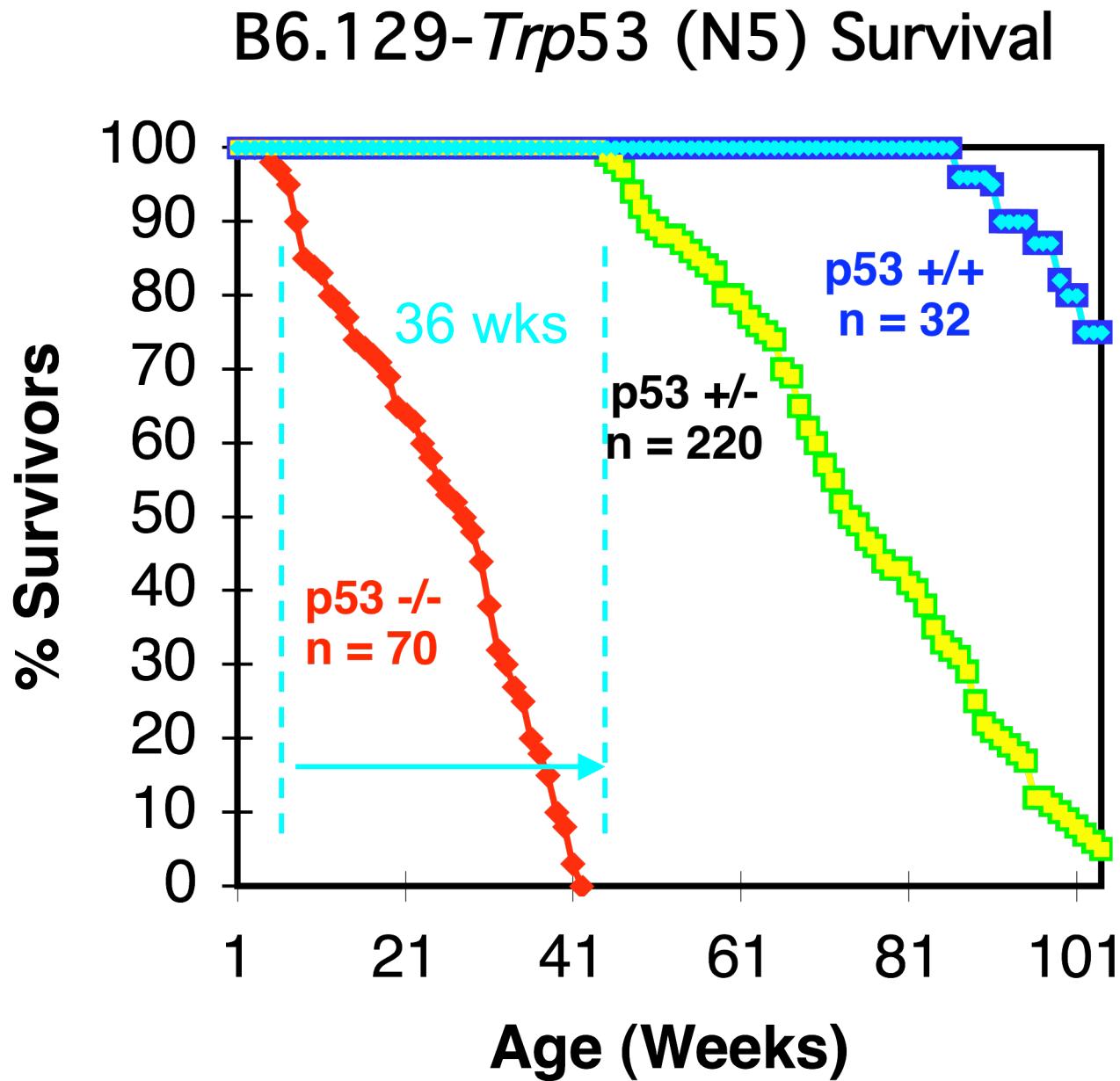
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Heterozygous p53 deficient Mice

Donehower et al., *Nature* 356:212, 1992





Loss of the wildtype p53 allele in short term cancer studies^a using haploinsufficient B6.129-Trp53 (N4-N5) mice. LOH = > 2 SD change in null/wt signal.

Chemical	Mut ^b	Route	F344/N		B6C3F ₁		het p53 def		
			Sex	Target	Sex	Target	Sex	Result	LOH
Benzene	-; +	G, I	M,F	mul; mul	M,F	mul ;mul	M	sarc; thy lym	13/16
p-Cresidine	+;-	F,G	M,F	bl, liv; bl	M,F	bl;bl, liv	M,F	ub, liv; ub	3/19
VCD ^c	+;nt	SP	M,F	sk; sk	M,F	sk, ov	M,F	sk;sk,ov	12/24
Phenolphthalein ^d	-;+	F	M,F	ad, kid; ad	M,F	thy, sar	F	thy lym	21/21
Mephalan	?	IP					M,F	thy lym	14/16
Foreign ^e							M,F	sarc	12/16
DMN ^f	+;+	G					M,F	sarc	17/22
DMBA/TPA	+;+	D			M,F	Sk	M,F	scc	10/16
Radiation (¹³⁷ Cs)	?;+	WB					M,F	thy lym	17/20
BBN ^h	+;?	G					M,F	ub	3/20

^a Replication of the NCI/NTP 2 year

^b Salmonella; in vivo micronucleus assay

^c 4-Vinyl-1-cyclohexene diepoxyde

^d Phenolphthalein

^e transponder microchip

^f Dimethylnitrosamine

^g linear energy transfer (cobalt 60)

^h N-butyl-N-(4- hydroxybutyl)nitrosamine



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Conclusions

- p53 haploinsufficient mice are susceptible to mutagenic carcinogens
- LOH includes both p53 locus specific and chromosome 11, and genomic gain/losses in gene copy number
- Induction of p53 mutations and/or LOH is carcinogen and tissue/organ specific



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Cdkn2a haploinsufficient

B6.129-Cdkn2a^{tm1Rdp} N2

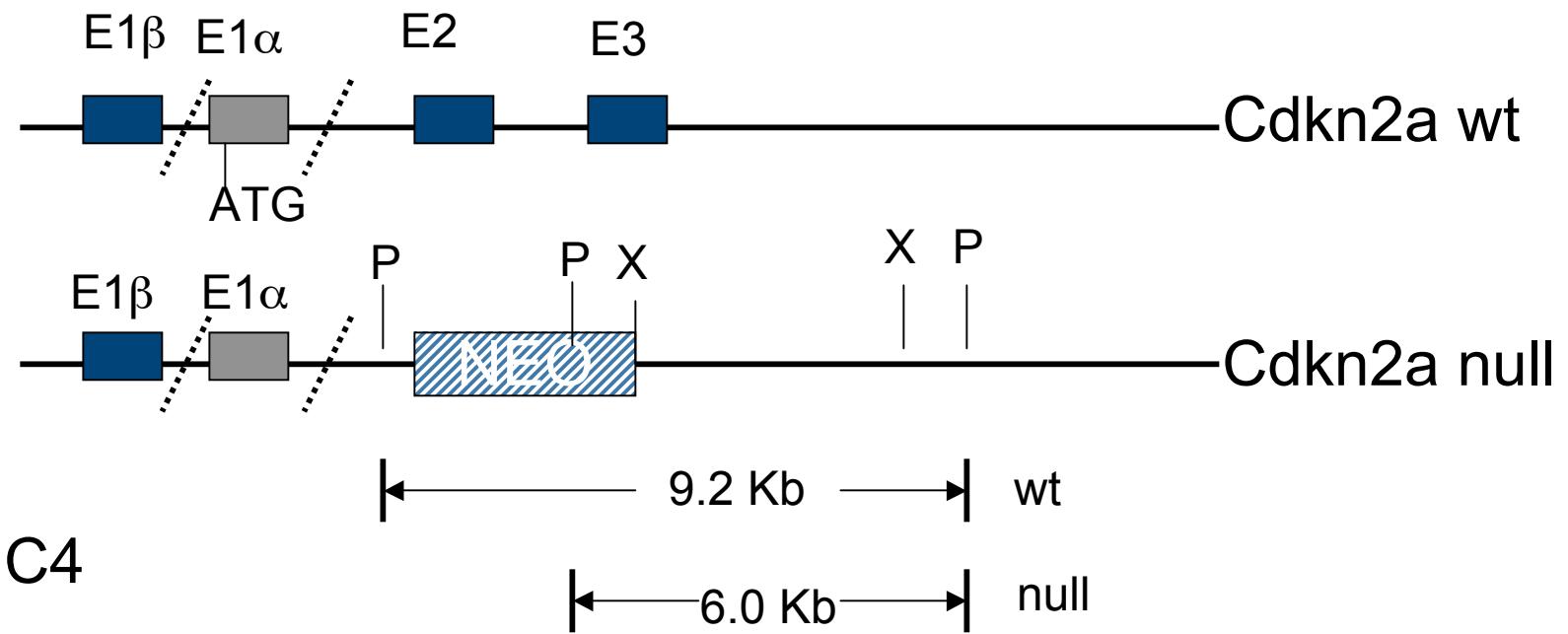




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Heterozygous *Cdkn2a* deficient mice



Serrano et al. Cell 85, 27-37, 1996



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Cdk2a: 1 locus, 2 genes (allelic variants) and 2 functions

- p16^{Ink4a} and p19^{Arf} - different promoters, E1α + E2 + E3 transcript = p16^{Ink4a}, E1β transcript + E2 + E3 = p19Arf
- **p16^{INK4a}** protein binds to CDK4 and 6 and prevents phosphorylation of RB (phosphorylation facilitates entry into the S phase) inhibiting the Cyclin D/CDK/Rb pathway and suppressing G1 to S transition
- **p19^{ARF}** protein (mouse)/P14ARF protein (human) binds with MDM2, targets p53 for degradation, thus, preventing MDM2 regulation of p53



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Cdkn2a (p16^{Ink4a}/p19^{Arf}) Mouse Model

- *Cdkn2a* (-/-) founders were derived from a C57BL/6 X WW6 embryo that was backcrossed twice to inbred C57BL/6 females (80% C57BL/6, 19% 129, and 1% SJL) to produce NTP mice
- Mice carry a null allele with a targeted deletion of the *Cdkn2a* locus that eliminates both p16^{Ink4a} and p19^{Arf}
- p16^{Ink4a}/p19^{Arf} (-/-) mice develop spontaneous tumors after week 30- fibrosarcoma, liposarcoma, lymphoma



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Conclusions

- Hypermethylation of $p16^{Ink4a}$ and /or $p19^{Arf}$ promoter sequence and/or allelic loss (LOH) at $Cdkn2a$ locus by carcinogens show both epigenetic and genetic mechanisms of carcinogenicity
- $p16^{Ink4a}/p19^{Arf}$ haploinsufficient mice are susceptible to mutagenic and non-mutagenic carcinogens

Mutagenic vs. Non-mutagenic Carcinogens in GMM

Model	Mutagen	Non-Mutagen
FVB/N-Tg.AC	✓	✓
p53 Deficient	✓	
p16/p19 Deficient	✓	✓



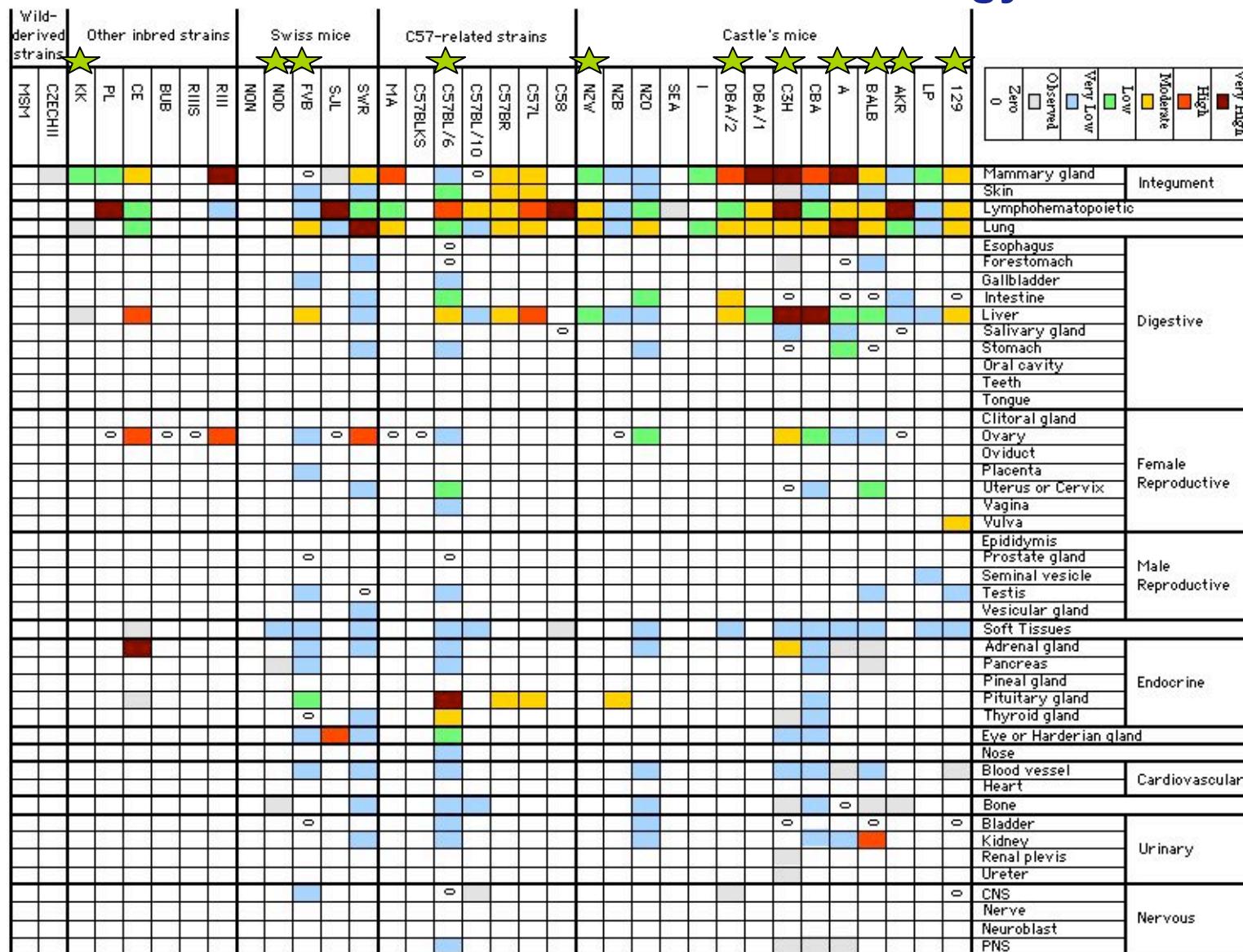
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Questions



*CAST/EiJ, BTBR T+/-J, MOLF/EiJ, PWD/PhJ, WSB/EiJ

Strain Tumor Biology



Legend: "Very High" - >80%-100%, "High" - >50% and 80%, "Moderate" - >20% and 50%, "Low" - >10% and 20%, "Very Low" - >0% and 10%, "Observed" - authors did not indicate frequency "Zero" - no reported tumors